

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



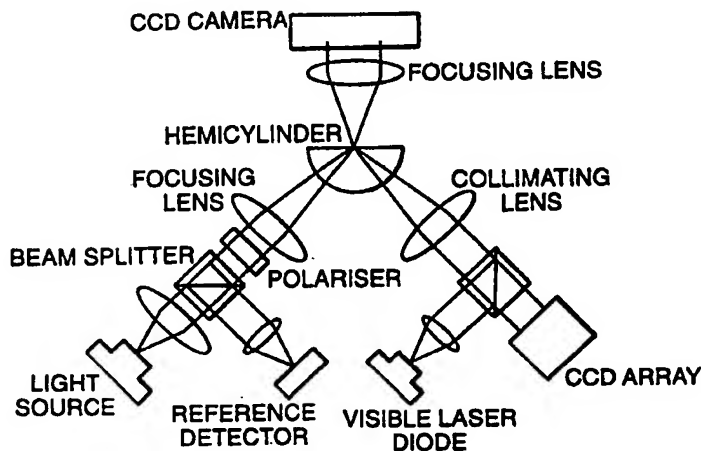
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : G01N 21/55		A1	(11) International Publication Number: WO 98/22808
			(43) International Publication Date: 28 May 1998 (28.05.98)
(21) International Application Number: PCT/GB97/03037		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 5 November 1997 (05.11.97)		<p>Published With international search report.</p>	
(30) Priority Data: 9623820.9 16 November 1996 (16.11.96) GB			
(71) Applicant (for all designated States except US): THE SECRETARY OF STATE FOR DEFENCE [GB/GB]; Defence Evaluation & Research Agency, Ively Road, Farnborough, Hampshire GU14 0LX (GB).			
(72) Inventor; and (75) Inventor/Applicant (for US only): PERKINS, Elaine, Ann [GB/GB]; CBDE, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB).			
(74) Agent: SKELTON, S., R.; D/IPR, Formalities Section (Procurement Executive), Poplar 2, MOD Abbey Wood #19, Bristol BS34 8JH (GB).			

(54) Title: ANALYTICAL APPARATUS

(57) Abstract

A surface plasmon resonance apparatus for detecting a soluble analyte (e.g. a protein) or a particulate analyte (e.g. a cell), the apparatus comprising: (a) a sensor block adapted to receive a sensor, said sensor, for example a sensor slide, having a metallised sensor surface capable of binding the analyte; (b) a light source capable of generating an evanescent wave at the sensor surface of a sensor slide on the sensor block; (c) a first detector capable of detecting light from the light source which is internally reflected from the sensor surface; and (d) a second detector (e.g. a video camera) capable of detecting light scattered or emitted from an analyte bound thereto. Optionally the apparatus further comprises a second light source for increasing the intensity of the light scattered or emitted from an analyte bound to the sensor surface, preferably this is sited such as to minimise the amount of light transmitted therefrom which is detected by the first detector. Also disclosed are sensors adapted for use in the apparatus, and methods of detecting analytes in samples comprising exposing samples to the sensor surface of the apparatus.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

ANALYTICAL APPARATUS

TECHNICAL FIELD

The present invention relates broadly to apparatus for the detection of analytes. The invention further relates to methods
5 employing such apparatus.

BACKGROUND ART

The use of Surface Plasmon Resonance (SPR) for the detection of small soluble analytes from solution is well known (see e.g. "Advances in Biosensors - A Research Annual Vol 1. 1991" Ed. A P F
10 Turner, Pub. Jai Press Ltd, London).

Briefly, an SPR apparatus generally comprises a light source for generating polarised light; a sensor, the outside of which is metal coated and may be contacted with a sample solution, and means for detecting the light which is internally reflected from
15 the inner sensor surface.

In the absence of bound analyte, light is totally internally reflected at an incident angle characteristic of the refractive index (RI) of the sensor and of the sample solution. At a particular incident angle (the 'SPR angle'), interaction of the
20 metal with the evanescent wave set up by internal reflection of the polarised light causes a drop in intensity of the reflected light. This drop can be observed using the light detector.

The binding of analyte to the sensor surface, within the evanescent wave zone, alters the RI of the sensor and this
25 perturbs the SPR angle. This perturbation can be observed using the light sensor and related to the surface concentration of analyte.

SPR detection in the literature has generally been limited to use with soluble molecular size analytes e.g. biomolecules such as
30 proteins and nucleic acids which are specifically bound within the evanescent zone using appropriate ligands.

However, the SPR apparatus in the art to date has not been suitable for accurately detecting sample materials with both soluble and insoluble analytes therein. In particular, due to the more limited way in which (for instance) roughly spherical cells of several μm diameter interact with the evanescent zone, only fairly high concentrations (e.g. $10^7 - 10^8/\text{ml}$) have been detectable using SPR. Thus in order to detect cells, as opposed to (for instance) protein antigens, further apparatus, and hence more cost, time and experimentation, have been required. For instance cells have frequently been detected using culture techniques followed by specific detection.

DISCLOSURE OF THE INVENTION

In the first aspect of the present invention there is disclosed a surface plasmon resonance apparatus for detecting a soluble and/or a particulate analyte, the apparatus comprising:

- (a) a sensor block adapted to receive a sensor, said sensor providing a metallised sensor surface capable of binding the analyte;
- (b) a light source capable of generating an evanescent wave at the sensor surface of a sensor on the sensor block;
- (c) a first detector capable of detecting light from the light source which is internally reflected from the sensor surface,
- (d) a second detector capable of detecting light scattered or emitted from an analyte bound at the sensor surface.

Suitable sensors are slides.

Possible analytes may include those particulate or insoluble analytes containing or consisting of biomolecules, for instance bacteria or other cells, spores, viruses or virions etc., or biomolecules themselves such as proteins or polynucleotides. Possible bacterial targets include *cryptosporidium*, *E. coli*, *salmonella* etc.

The apparatus may thus be used with a wide variety of samples suspected or known to contain analytes. For examples environmental samples such as water, or biological samples.

Broadly speaking the apparatus operates as follows: in use the
5 first detector detects the binding of soluble analytes to the sensor surface by detecting the changes in the intensity of light internally reflected from the sensor surface, whereas the second detector detects the binding of particulate analytes to the sensor surface by detecting the light scattered or emitted from the
10 analytes bound thereto. The apparatus of the present invention is therefore capable of the sensitive detection of both soluble and particulate analytes, and thus may provide a quicker, cheaper or more sensitive alternative to the methods and apparatus presently used in the art.

15 It is important to stress the different functions of the detectors in the apparatus. The first detector must be arranged to detect light internally reflected from the sensor surface, the intensity of this light being dependent on the SPR effects occurring as analytes (especially soluble ones) bind at the sensor surface
20 altering the refractive index of the sensor/sample interface. The detector may be a 2-D array detector as described in more detail in the Examples below.

By contrast the second detector detects light which is scattered or otherwise emitted (optionally by fluorescence) from analytes
25 (especially particulate ones) which interact with the evanescent field at the sensor surface. This may give a sensitivity for detecting large particulate analytes several orders of magnitude higher than would be obtainable using pure SPR. Clearly the nature of the second detector used will determine the sensitivity and
30 acuity of the detection, but in preferred embodiments single cells bound within the evanescence zone may be detected and resolved using the second detector while the bulk binding effects of soluble molecules may be detected using the first.

Preferably the second detector is a video camera (e.g. a Charge
35 Coupled Detector [CCD] camera), but any kind of light detector appropriated for detecting light scattered or emitted from the

analytes may be used e.g. a 2-D diode array, a photomultiplier etc.

In one embodiment of the first aspect, the second detector is located on the same side of the surface as the light source such as to be capable of detecting light which is back-scattered or emitted when an analyte is bound to thereto.

The term 'light source' as used herein means any source of light radiation, including where appropriate the tip of an optical fibre which is attached to a remote radiation source.

In a different embodiment, the second detector is located on the opposite side of the surface as the light source detector such as to be capable of detecting light which is scattered or emitted when an analyte is bound to thereto

In either case it may be desirable that the second detector is located such as to be capable of detecting light scattered or emitted at a predetermined angle, for example substantially normally, to the sensor surface. This will minimise interference from light which is being totally internally reflected from the surface.

Generally the sensor block will comprise a prism or a hemicylinder, such as are known to those skilled in the art of SPR detection. The sensor block is adapted to receive the detachable sensor which provides the metallised surface. The adaptation may simply consist of providing a general area to mount the sensor such as a slide, or the block may be specially shaped or configured to receive it e.g. in a groove or properly-dimensioned well.

The block and or sensor may in addition be adapted to form all or part of one wall of a flow channel, through which a liquid sample can flow in liquid contact with the metallised surface. An apparatus comprising such a flow channel forms one embodiment of the first aspect of the invention.

Preferably the metallised sensor surface is adapted or otherwise functionalised such as to facilitate the immobilisation of

macromolecules which are capable of specifically binding biomolecules thereto. For instance the sensor may have a hydrophilic dextran surface. Antibodies may then be immobilised thereto in order to specifically bind antigenic analytes.

5 Alternatively a polynucleotide probe may be immobilised for specifically binding a polynucleotide analytes.

Preferably the e.g. antibodies are bound only to discrete portions of surface in order to facilitate the detecting light which is scattered or emitted when an analyte is bound to thereto. These
10 portions may then be visualised (and possibly further resolved) by the second detector as contrasting discrete bright areas against the darker portions of the surface which do not have macromolecules bound to them.

The surface may have greater than one type of macromolecule
15 immobilised thereto for specifically binding greater than one type of antigen. The different types of e.g. antibody may be bound in known discrete areas in order to easily identify which antigen is being specifically bound.

In one further embodiment of the invention, the apparatus includes
20 a second light source. This can be used to increase the intensity of the light scattered or emitted from the sensor surface when an analyte is bound thereto. Although this embodiment requires additional components, it has the advantage that the light source can be optimised (e.g. wavelength, angle of incidence against the
25 sensor surface, intensity) for light scattering and/or fluorescence.

It may be desirable to locate the second light source such as to minimise the amount of stray light emitted therefrom which is detected by the first detector.

30 This may be done by locating the second light source such that light emitted therefrom travels along the same light path but in the opposite direction from the light from the first light source which is internally reflected from the sensor surface to the first detector, as is shown in the Figures below.

The light source(s) used can be selected without undue burden by those skilled in the art. In order to maximise intensity, and hence sensitivity, the or each light source may be a laser light source, or a light emitting diode.

- 5 In a second aspect of the invention there is disclosed a method of detecting an analyte in a sample comprising exposing the sensor surface of an apparatus as described above to the sample. The analyte may then be detected by the first or second detector.

For instance a soluble analyte in a sample may be detected by
10 detecting the changes in the intensity of light internally reflected from the sensor surface. A particulate analyte in a sample may be detected by detecting the light scattered or emitted from the analytes bound to the sensor surface. Preferably the apparatus is arranged such that soluble or particulate analytes
15 may be detected simultaneously.

In a third aspect of the invention there is disclosed a surface plasmon resonance apparatus for detecting an analyte, the apparatus comprising:

- (a) a sensor block adapted to receive a sensor, said sensor having
20 a metallised sensor surface capable of binding the analyte;
- (b) a light source capable of generating an evanescent wave at the sensor surface of a sensor on the sensor block;
- (c) a first detector capable of detecting light from the light source which is internally reflected from the sensor surface,
- 25 (d) means adapted to secure a second detector capable of detecting light scattered or emitted from an analyte bound at the sensor surface.

The apparatus of third aspect has the advantage that it may be used to readily construct the apparatus of the first aspect. In
30 particular. The means adapted to secure the second detector may comprise a holder or clamp positioned and/or dimensioned to receive e.g. a video camera and associated optics, such that it can detect light scattered or emitted from the sensor surface. The

holder or clamp may be moveable in a pre-determined way to facilitate the function of the second detector when in place e.g. to allow focusing.

5 Preferably the means are adapted to secure the second detector such that it is capable of detecting light emitted at a predetermined angle, for example substantially normally, to the sensor surface.

10 The first detector of the apparatus may also be adapted such as to receive a second light source. The adaptation may be such that the second light source, when in place, is configured to minimise interference with the first detector by being directed away from it, as described above.

15 A fourth aspect of the invention embraces a second detector adapted (e.g. by its construction and dimensioning) for use in the apparatus of the third aspect.

20 A fifth aspect is a sensor having a metallised surface and being adapted for the apparatus above, in particular so as to allow light emitted or scattered from the sensor surface to be transmitted to the second detector. The sensor may comprise a slide and the surface may be functionalised in discrete sections as described above.

FIGURES

25 Fig 1 Shows a schematic diagram of a surface plasmon resonance apparatus for detecting a soluble or a particulate analyte, as described in more detail in Example 1.

Fig 2 Shows a block diagram of the complete instrument of Example 1.

30 Fig 3 Shows how the apparatus may be used to detect multiple analytes. Fig 3(a) and (b) show the light source, hemicylinder (plus detection surface), and CCD array detector schematically. Fig 3(c) shows a detail of the CCD array.

Fig 4 Shows bound particles scattering light from the metallised detection surface of a hemicylinder sensor. The light can be detected by a video camera (not shown).

Fig 5 shows scattering from bacterial particles above a silver surface: the points of light represent scattered light from *Erwinia herbicola*.

EXAMPLES

EXAMPLE 1: SURFACE PLASMON RESONANCE APPARATUS FOR DETECTING A SOLUBLE OR A PARTICULATE ANALYTE

Fig 1 Shows a schematic diagram of a surface plasmon resonance apparatus for detecting a soluble or a particulate analyte, such as could be constructed (in the light of the present disclosure) by those skilled in the art. A block diagram of the components of the apparatus is shown in Fig 2.

This system may be rearranged if desired, for instance the polariser may be placed after the hemicylinder if required.

Considering Fig 1, the light path to the first detector ('CCD Array') is from the light source at the left, through the beam splitter (which splits a portion to the reference detector), through a polariser and focusing lens, off the internal surface of the hemicylinder, through a collimating lens and into the CCD array.

The light path is shown schematically in Fig 3(a). An extended collimated source may be used to illuminate the hemicylinder surface continuously over a range of incident angles, as shown in Fig 3(b). The CCD array is composed of a pixelated array of individual light sensors, each detecting a different reflected angle or being used to detect a different sample analyte (in this case 4 different samples) as shown in Fig 3(c). This allows the rapid monitoring without moving parts.

Considering Fig 1, the light path to the second detector ('CCD camera') is from the light source at the left, through the beam splitter (which splits a portion to the reference detector), through a polariser and focusing lens and onto the hemicylinder.

5 The intensity is supplemented in this embodiment by light from the visible laser diode on the right which travels away from the CCD array and through the collimating lens on the right and onto the hemicylinder. The evanescent field generated on the upper, metallised, surface of the hemicylinder causes particles bound
10 therein to scatter light as depicted in Fig 4. The scattered light is focused through a lens and detected by the CCD camera.

Naturally if the particles were fluorescently labelled, using reagents (e.g. fluourescein) and methods well known to those skilled in the art, then the CCD camera could detect emitted light
15 as the particles are excited by the evanescent field.

Devices according to Example 1 may be constructed based on existing SPR machines but having the additional components described above. The machines and components may be those available commercially. For instance the light source may
20 advantageously be an edge emitting LED as used in fibre-optic communications (e.g. EG&G type S86018). A stabilised power supply may be used to minimise artefacts.

The sensor may be metal-coated microscope slide (or similar thickness dielectric) which is index matched onto the hemicylinder
25 with fluid of similar refractive index. A portion of the hemicylinder may be ground off to accommodate the slide.

The CCD array (with 'pixels' about $20 \mu\text{m}^2$) may be of a type developed for video use. Readout from CCD was accomplished by transferring a sample-area row to a readout or row register.
30 Correlated Double Sampling (CDS) may be used to eliminate noise. The analog output can be passed to a digital signal processor via an ADC. A suitable processor is an Analog Devices ADSP-2105. This can communicate with an external host PC via a bi-directional parallel port.

The CCD video camera can be a conventional, commercially available, one e.g. as sold by Hamamatsu (Japan).

EXAMPLE 2: METHOD OF USE OF SURFACE PLASMON RESONANCE APPARATUS

5 In use, in order to correct for differences in source intensity along the collimated beam, a calibration can be carried out before the experiment. The sensor surface is then exposed to the sample(s). The host selects monitoring angles through using reflectivity vs. angle scans. Data is then acquired over a set time period and displayed by the host PC.

10

Example 3

Detection of particulate analyte using the second detector

15 In order to illustrate the light scattering technique, a glass microscope slide was coated with silver for optimum surface plasmon resonance (48nm). The slide was then mounted onto a glass hemicylindrical prism and illuminated with a 3mW helium-neon laser. The slide was covered with a film of bacteria (*Erwinia herbicola*) at 1×10^6 /ml in phosphate buffered saline solution. The bacteria were then allowed to adsorb onto the surface of the
20 silver microscope slide.

The bacteria were then allowed to adsorb onto the surface of the silver microscope slide. The output from the CCD array above the SPR surface is a normal video output with 256 levels of brightness. Observation above the silver surface showed that
25 initially all pixels on the CCD camera gave a low reading (1-20) and the surface appeared dark. As the bacteria approached the surface, the brightness increased for those pixels specifically aligned with the areas where the bacteria were on the surface. The maximum brightness level recorded from the light scattered by
30 the bacteria at the surface was 230. The appearance of the surface was that of a dark background with bright spots associated with the bacteria on the surface (See Figure 5).

As a control, a film of phosphate buffered saline without bacteria was used to cover the silver surface of a similar microscope slide. This time, no scattering from the surface was observed.

CLAIMS

1. A surface plasmon resonance apparatus for detecting a soluble or a particulate analyte, the apparatus comprising: (a) a sensor block adapted to receive a sensor, said sensor providing a metallised surface capable of binding the analyte; (b) a light source capable of generating an evanescent wave at the sensor surface when positioned on the sensor block; (c) a first detector capable of detecting light from the light source which is internally reflected from the said metallised surface; characterised in that the apparatus further comprises: (d) a second detector capable of detecting light scattered or emitted from an analyte bound at the sensor surface.
2. An apparatus as claimed in claim 1 wherein the particulate analyte is selected from the list comprising: a procaryotic cell; a eucaryotic cell; a virus or virion.
3. An apparatus as claimed in claim 1 or claim 2 wherein the soluble analyte is a biomolecule selected from the list comprising: proteins; nucleic acids.
4. An apparatus as claimed in any one of the preceding claims wherein the second detector is a video camera
5. An apparatus as claimed in any one of the preceding claims wherein the second detector is located on the same side of the sensor surface as the light source such as to be capable of detecting light which is back-scattered or emitted when an analyte is bound to thereto
6. An apparatus as claimed in any one of claims 1 to 4 wherein the second detector is located on the opposite side of the surface as the light source such as to be capable of detecting light which is scattered or emitted when an analyte is bound to thereto
7. An apparatus as claimed in claim 5 or claim 6 wherein the second detector is located such as to be capable of detecting

light scattered or emitted at a predetermined angle to the sensor surface.

8. An apparatus as claimed in any one of the preceding claims wherein the sensor block defines all or part of a flow channel through which a liquid sample can flow.
9. An apparatus as claimed in any one of the preceding claims further characterised in that it comprises a second light source for increasing the intensity of the light scattered or emitted from an analyte bound to the sensor surface.
10. An apparatus as claimed in claim 9 wherein the second light source is located such as to minimise the amount of light transmitted therefrom which is detected by the first detector
11. An apparatus as claimed in claim 10 wherein the second light source is located such that light transmitted therefrom travels along the same light path but in the opposite direction from the light from the first light source which is internally reflected from the sensor surface to the first detector.
12. An apparatus as claimed in any one of claims 9 to 11 wherein the second light source transmits light of a different wavelength to the first light source
13. An apparatus as claimed in any one of the preceding claims wherein the or each light source is a laser light source
14. An apparatus as claimed in any one of the preceding claims wherein the or each light source is a light emitting diode
15. An apparatus according to any one of the preceding claims wherein the sensor block is adapted to receive a sensor slide.
16. A surface plasmon resonance apparatus for detecting an analyte, the apparatus comprising: (a) a sensor block adapted to receive a sensor, said sensor having a metallised sensor surface capable of binding the analyte; (b) a light source capable of

generating an evanescent wave at the said metallised sensor surface when the sensor is present on the sensor block; (c) a first detector capable of detecting light from the light source which is internally reflected from the sensor surface, characterised in that the apparatus further comprises (d) means adapted to secure a second detector capable of detecting light scattered or emitted from an analyte bound at the sensor surface.

17. An apparatus as claimed in claim 16 being adapted to secure the second detector such that it is capable of detecting light scattered or emitted substantially normally to the sensor surface.

18. A second detector adapted to be secured by the apparatus of claim 16 or claim 17 such that it is capable of detecting light scattered or emitted from an analyte bound at the sensor surface.

19. A sensor adapted for use in the apparatus of any one of the preceding claims.

20. A sensor according to claim 19 which comprises a slide.

21. A sensor as claimed in claim 19 or 20 having a hydrophilic dextran surface in order to facilitate the immobilisation of macromolecules thereto which are capable of specifically binding biomolecules.

22. A sensor as claimed in any one of claims 19 to 21 wherein the sensor is adapted such that in use it defines all or part of a flow channel through which a liquid sample can flow.

23. A sensor as claimed in any one of claims 19 to 22 having antibodies immobilised to the surface thereof for specifically binding an antigenic analyte.

24. A sensor as claimed in claim 23 wherein the antibodies are bound only to discrete portions of surface in order to facilitate the detecting light which is scattered or emitted when an analyte is bound to thereto

25. A sensor as claimed in claim 23 wherein the surface has more than one type of antibody immobilised thereto for specifically binding more than one type of antigen.

26. An apparatus as claimed in any one of claims 1-17 further comprising a sensor.

27. An apparatus as claimed in claim 24 wherein the sensor is the sensor of claims 18 to 23.

28. A method of detecting an analyte in a sample comprising exposing sample to the sensor surface of the apparatus of claim 26 or 27.

29. A method of detecting a soluble analyte in a sample comprising use of the method of claim 28 wherein changes in the intensity of light internally reflected from the sensor surface are detected.

30. A method of detecting a particulate analyte in a sample comprising use of the method of claim 28 wherein the light scattered or emitted from the analytes bound to the sensor surface is detected.

31. A method as claimed in any one of claims 28 to 30 wherein soluble and particulate analytes are detected simultaneously.

32. An apparatus as claimed in any one of claims 1 to 18 or claims 26 or claim 27 substantially as described herein with reference to the drawings.

Fig.1.

1/3

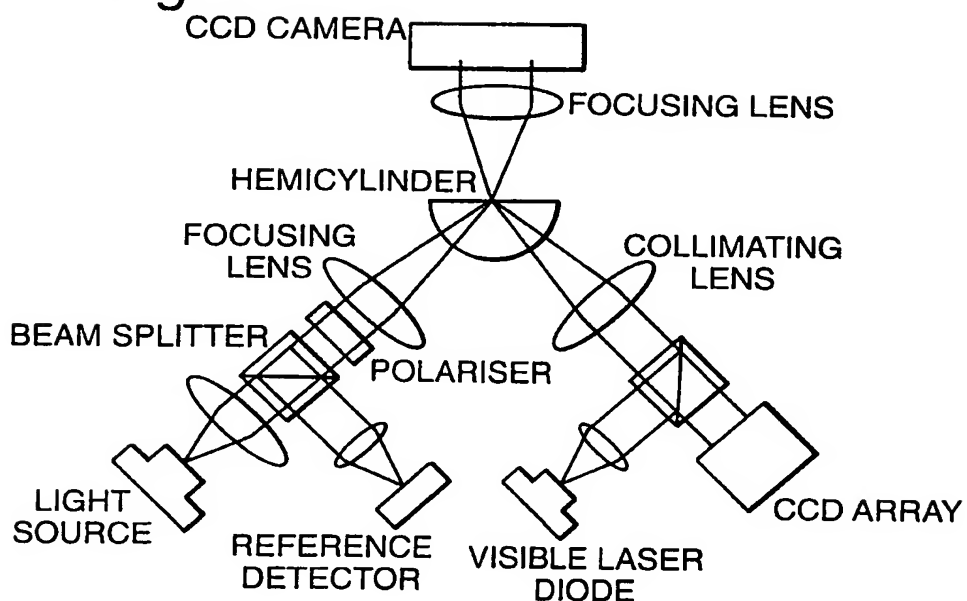


Fig.2.

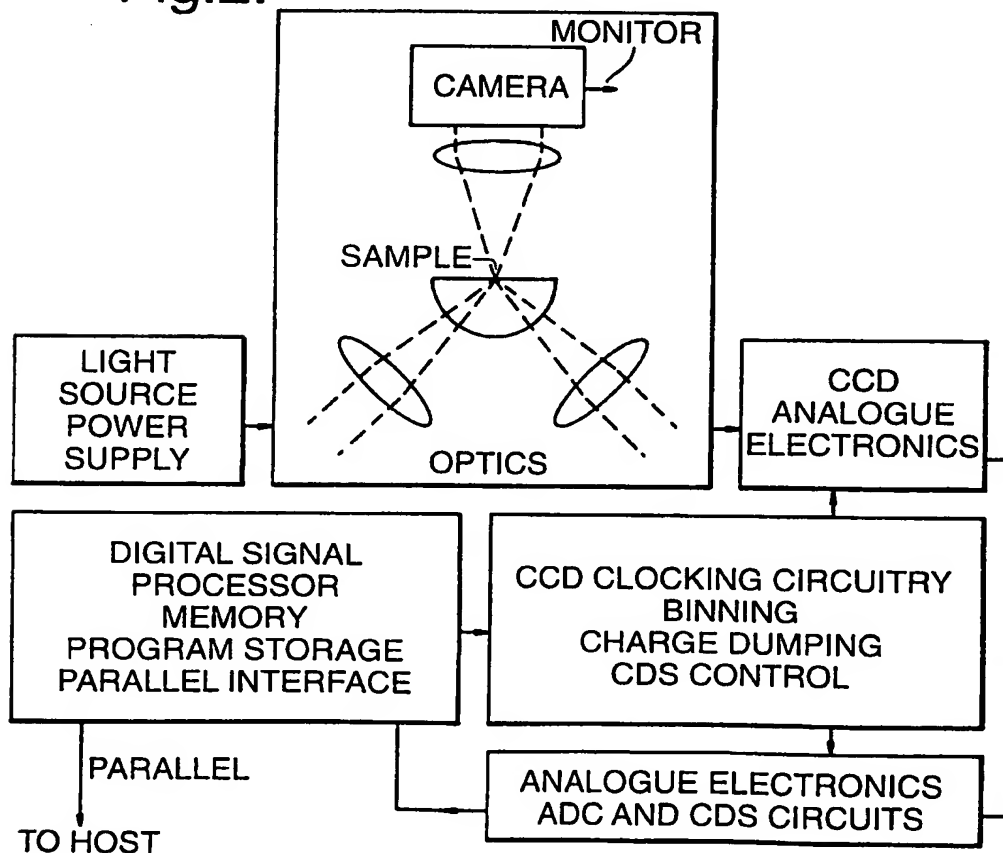


Fig.3(a).

2/3

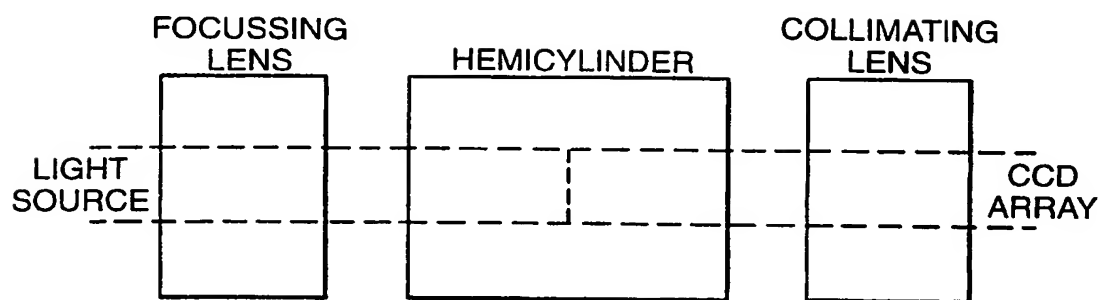


Fig.3(b).

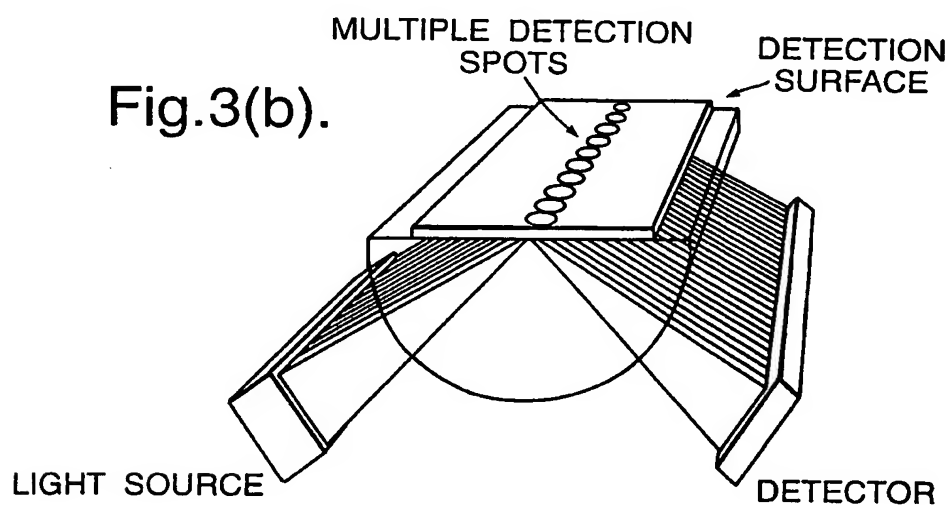
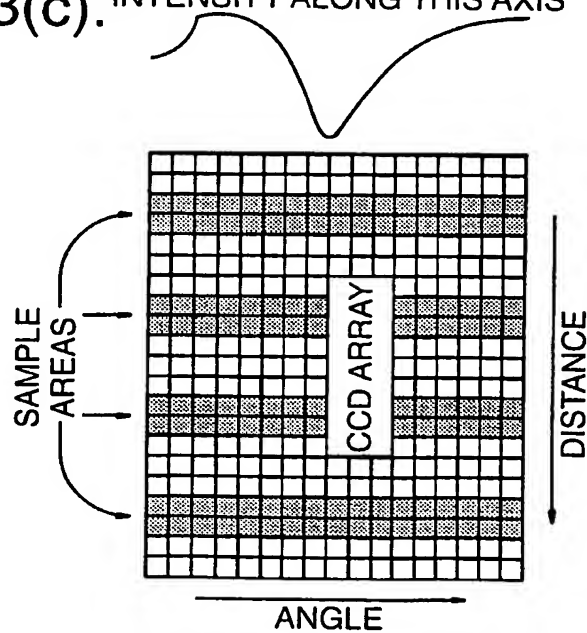


Fig.3(c). INTENSITY ALONG THIS AXIS



3/3

Fig.4.

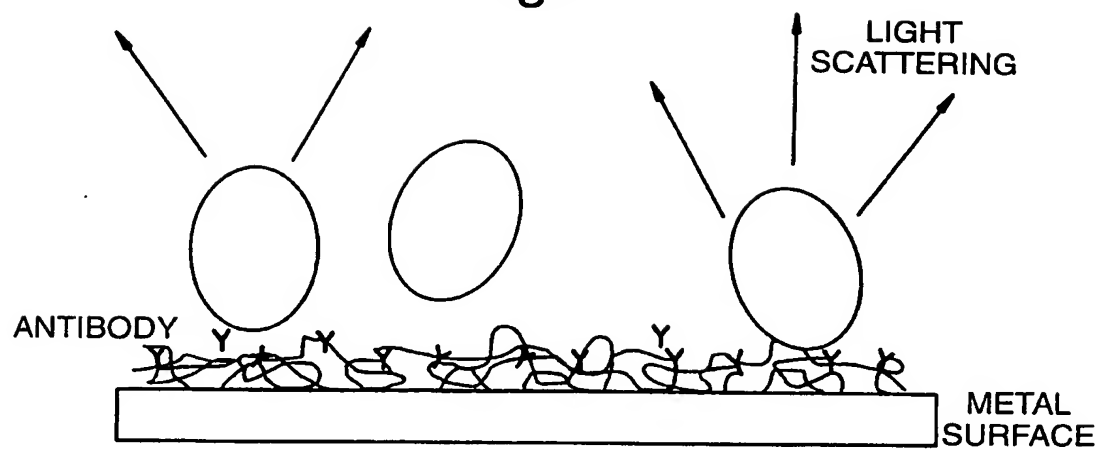
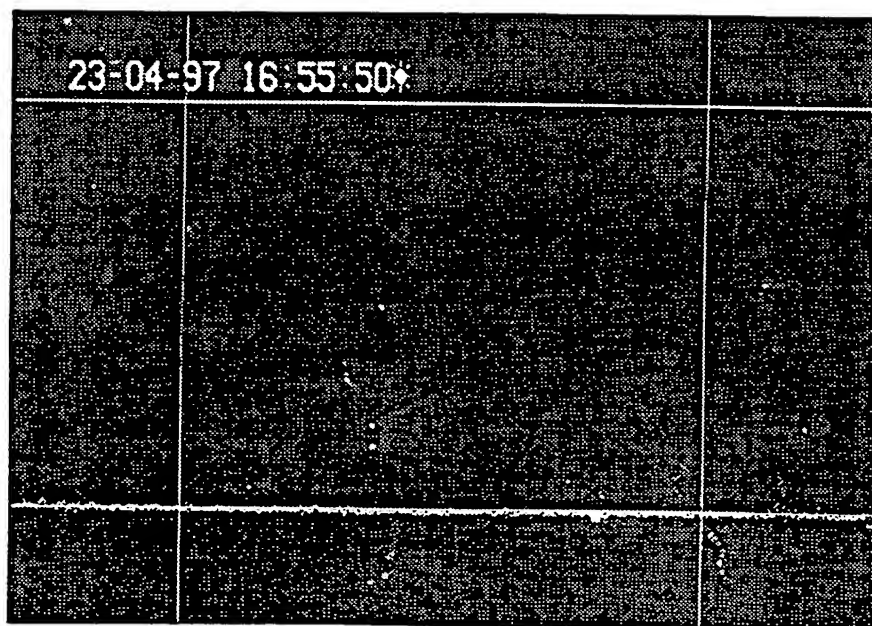


Fig.5.



INTERNATIONAL SEARCH REPORT

Inter. Appl. Application No
PCT/GB 97/03037

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N21/55

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 05295 A (PHARMACIA AB) 17 May 1990	1,3,5,7, 8,13-32
Y	see page 15, line 19 - page 16, line 6 see page 17, line 16 - page 18, line 30 see page 21, line 29 - page 22, line 28 see page 25, line 27 - page 26, line 9 see page 32, line 5 - line 23 see page 35, line 22 - page 36, line 6; figures 1,4	2,4,6,9, 12
Y	--- US 5 485 277 A (FOSTER MARK W) 16 January 1996	2,4
A	see column 4, line 25 - line 40 see column 5, line 45 - column 6, line 22 see column 13, line 20 - line 26 --- -/-	1

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

5 February 1998

Date of mailing of the international search report

25.02.98

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2260 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Navas Montero, E

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/03037

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 437 840 A (KING DAVID A ET AL) 1 August 1995	6
A	see column 11, line 33 - line 68; figure 3 ---	1
Y	PETERLINZ K A ET AL: "TWO-COLOR APPROACH FOR DETERMINATION OF THICKNESS AND DIELECTRIC CONSTANT OF THIN FILMS USING SURFACE PLASMON RESONANCE SPECTROSCOPY" OPTICS COMMUNICATIONS, vol. 130, no. 4/06, 1 October 1996, pages 260-266, XP000627753	9,12
A	see figure 2 -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 97/03037

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9005295 A	17-05-90	SE 462408 B DE 68912343 D DE 68912343 T EP 0534941 A EP 0442921 A JP 4504765 T JP 4501462 T SE 8804075 A WO 9005317 A US 5164589 A US 5313264 A	18-06-90 24-02-94 05-05-94 07-04-93 28-08-91 20-08-92 12-03-92 10-11-88 17-05-90 17-11-92 17-05-94
US 5485277 A	16-01-96	NONE	
US 5437840 A	01-08-95	EP 0677735 A JP 7311146 A US 5514596 A	18-10-95 28-11-95 07-05-96